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Complete Interference and Nonrandom Distribution of Meiotic Crossover in a Mollusc, *Mulinia lateralis* (Say)

XIMING GUO AND STANDISH K. ALLEN, JR.

Haskin Shellfish Research Laboratory, Institute of Marine and Coastal Sciences, Rutgers University, Box B-8, Port Norris, New Jersey 08349

In gene mapping, the genetic distance between two genes is measured by the frequency of meiotic crossovers occurring between them. It is generally assumed that there is more than one crossover per chromosome, and the distribution of crossovers along chromosomes is random and follows a Poisson distribution (no interference), or that interference is inversely correlated with distance. Although those assumptions may be correct for some organisms, we report here a novel exception in the marine mollusc Mulinia lateralis *Say. Using segregation analysis of gynogenetic diploids, we found surprisingly high gene-centromere recombinant frequencies for most of the 13 allozyme loci studied. For at least six loci, there was always one and only one crossover occurring between the gene and its centromere, suggesting complete interference where the occurrence of one crossover completely suppressed the occurrence of another. The complete interference was confirmed by the cytogenetic observation that there was only one chiasma for all bivalents. Further, sites of the single crossover seem not to be randomly distributed along chromosomes, but preferentially located in a recombination hot-region proximal to the centromere. The restricted distribution of a single crossover per chromosome provides one explanation for the unique phenomenon of heterozygote deficiency in* M. lateralis *and other molluscs.*

During meiosis, homologous chromosomes—one maternal and one paternal—duplicate and pair up, forming a bivalent consisting of four chromatids. Genetic recombination occurs when two homologous chromatids cross over. Meiotic crossovers are thought to occur randomly along the chromosome, and their frequencies are correlated with physical DNA distances. In gene mapping, therefore, the frequency of crossovers (or recombinants) is used to estimate genetic distance. Gene mapping becomes complicated under crossover interference, where the occurrence of one crossover affects the chance of another one occurring nearby. Although the importance of modeling crossover interference has recently been recognized in gene mapping (1–3), our basic understanding of meiotic crossover and interference remains limited.

Gene-centromere recombination analysis provides a unique opportunity to study meiotic crossover. Because every gene is known to have linkage to a centromere, gene-centromere crossover can be reliably analyzed over a long distance. Linkage analysis of two genes, on the other hand, is difficult across long distance. Gene-centromere mapping is possible in a few species of fungi and algae through the analysis of unseparated meiotic products such as tetrads (4). In organisms such as fish and molluscs, viable gynogenetic diploids, equivalent to half-tetrads, can be produced and used for the purpose of gene-centromere mapping (5–7). Previously, we produced meiotic gynogens in *M. lateralis* by inhibiting the second meiotic division in gynogenetically activated eggs (8). In this study, the gynogenetic diploids along with their triploid controls were used to investigate gene-centromere recombination of allozyme loci.

The success of gynogenesis was determined by the absence of paternal alleles in gynogenetic offspring. In all gynogens analyzed from three families, none of the diagnostic paternal alleles (8 to 11 per family) were found, suggesting that gynogenesis was complete. In normal diploids and triploids, paternal alleles were present as expected from Mendelian segregation.

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Thirteen allozyme loci were heterozygous in maternal parents, and their centromere recombinant frequencies (CR) were determined as the percentage of maternal heterozygotes among gynogenetic and triploid offspring. Segregation data from triploids were used only for loci whose paternal contribution could be clearly distinguished. Surprisingly high CR values were observed for most loci (Table I). The high CR values (or proportion of heterozygotes) cannot be explained by reduced survival of gynogens homozygous for recessive lethal genes. Triploids, which should be unaffected by recessive lethals, gave almost identical CR estimates as gynogens in all cases. Therefore, the high CR values must be a true reflection of high crossover frequencies between allozyme loci and their centromeres. A CR value of 100% means that there was always one and only one crossover occurring between the gene and its centromere, which is complete interference by definition; *i.e.*, the occurrence of the one crossover completely inhibits the occurrence of another one. Among the 13 loci, at least six, AAT, ALAT2, GPI, EST, MPI, and PGDH, exhibited complete interference. Another four loci, ALAT1, LAP, PGM, and TAP3, had high levels of interference with CR values higher than 95%, although complete interference could not be ruled out. Complete interference for all chromosomes is confirmed by cytogenetic data. *M. lateralis* has a haploid number of 19 chromosomes, all of which are telocentric (8, 11). For 16 meioses examined in eggs, all 304 (16 × 19) synapsed bivalents had a single chiasma (Fig. 1). Previously published photographs of meiotic chromosomes also clearly demonstrated one chiasma per bivalent (11). Although positive interference has been considered the rule for eukaryotes, most species have more than one crossover per bivalent and only moderate levels of interference; complete interference is rare (12–14). The telocentric nature of *M. lateralis* chromosomes is probably not the reason for only one chiasma per chromosome. Telocentric chromosomes of mice frequently have two chiasmata per chromosome (15). Because all parents were randomly selected from one base population, crossover inhibition by polymorphic inversions was also unlikely in this study.

Knowing the level of interference is crucial to the accuracy of gene mapping. In mapping analysis, it is generally assumed that there is more than one crossover per chromosome, and the distribution of crossovers is random and follows a Poisson distribution (no interference), or that interference is inversely correlated with genetic distance (16, 17). When there is complete or high levels of interference over the whole chromosome, as suggested by this study, those assumptions become invalid, and most models of map functions will inflate genetic distances. Under complete interference in *M. lateralis*, for example, a genetic distance of 20 cM would be inflated

Table I

Gene-centromere recombinant frequency (CR) of 13 allozyme loci estimated from gynogenetic diploid (Gyno) and triploid (3n) Mulinia lateralis

Loci		Offspring Genotype AA	AB	BB	CR (%)	Distance (cM)
AH2	Gyno	101	1	75		
	3n	26	2	27		
	Total	127	3	102	1.3	0.6
AH1	Gyno	15	27	13		
	3n	17	29	8		
	Total	32	56	21	51.4	25.7
DAP	Gyno	18	82	18	69.5	34.7
PGM	Gyno	3	111	3		
	3n	3	105	1		
	Total	6	216	4	95.6	47.8
ALAT1	Gyno	3	169	4	96.0	48.0
TAP3	Gyno	1	168	3		
	3n	1	78	1		
	Total	2	246	4	97.6	48.8
LAP	Gyno	1	117	0		
	3n	0	55	0		
	Total	1	172	0	99.4	49.7
AAT	Gyno	0	118	0		
	3n	0	71	0		
	Total	0	189	0	100.0	50.0
ALAT2	Gyno	0	117	0		
	3n	0	55	0		
	Total	0	172	0	100.0	50.0
GPI	Gyno	0	119	0	100.0	50.0
EST	Gyno	0	119	0		
	3n	0	52	0		
	Total	0	171	0	100.0	50.0
MPI	Gyno	0	178	0		
	3n	0	106	0		
	Total	0	284	0	100.0	50.0
PGDH	Gyno	0	118	0		
	3n	0	109	0		
	Total	0	227	0	100.0	50.0

Gene-centromere recombinant frequency (CR) was calculated as the portion of maternal heterozygotes among offspring (6, 7). CR estimates from gynogens and triploids of three families were combined for each locus because they did not differ significantly. Genetic distance was calculated by halving the CR values under the assumption of complete interference (4). Allozyme analysis of adult clams was conducted by starch gel electrophoresis (9, 10). Names of the allozymic loci were abbreviated as: AAT for asparate aminotransferase (E.C. 2.6.1.1), AH for aconitate hydratase (E.C. 4.2.1.3), ALAT for alanine aminotransferase (E.C. 2.6.1.2), DAP for dipeptidase (E.C. 3.4.*.*Gly-Leu as substrate), EST for esterase (E.C. 3.1.1.1), GPI for glucose-6-phosphate isomerase (E.C. 5.3.1.9), LAP for leucine aminopeptidase (E.C. 3.4.*.*), MPI for mannose-6-phosphate isomerase (E.C. 5.3.1.8), PGDH for phosphogluconate dehydrogenase (E.C. 1.1.1.44), PGM for phosphoglucomutase (E.C. 5.4.2.2), TAP for tripeptidase (E.C. 3.4.*.*Leu-Gly-Gly as substrate). AAT, ALAT2 and GPI were closely linked without double-crossover occurring among them (Guo *et al.*, in prep).

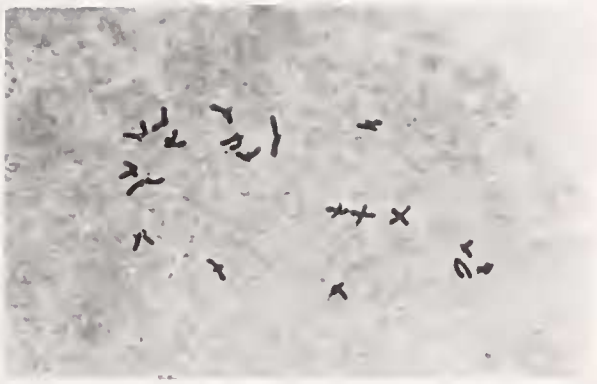

Figure 1. Chiasma formation in eggs of the dwarf surfclam, *Mulinia lateralis*, showing "V" or "X" shaped bivalents with only one chiasma. If there were two or three crossovers per chromosome, bivalents would have appeared in the shapes of "O" or "8" respectively. The single chiasma usually formed near the centromere ("V" shaped), except for 2 or 3 bivalents. Eggs were fixed in 3:1 methanol and acetic acid and stained with an acetic orcein stain.

by 6% with Kosambi's map function and by 28% with Haldane's function, and the inflation increases dramatically for longer distances.

The agreement between the finding of one crossover per chromosome through segregation analysis (at six loci) and the cytogenetic observation of one chiasma per chromosome weakens the chiasma reduction hypothesis. The chiasma reduction hypothesis states that some chiasmata may be eliminated prior to metaphase, and the observed chiasma frequency is an underestimate of actual recombination events, causing the chiasma-based maps to be shorter than recombination-based maps (18, 19). Our results indicate that there was no chiasma reduction—one recombination corresponds directly to one chiasma—for at least four of the 19 chromosomes in *M. lateralis* (AAT, GPI, ALAT2 are linked). For four other chromosomes, chiasma reduction, if any, must be small, because the chance that two recombinations occurred is less than 5% according to the CR values. It is true that recombination maps are usually longer than chiasma maps (18, 20). This discrepancy is likely caused by the inflation of the linkage map due to the underestimation of interference as discussed earlier, and not by chiasma reduction.

Another unexpected finding was that the CR values of the 13 loci seem not to be randomly distributed between 0 and 100 (Table I). Ten of the 13 loci (77%) had a CR of 95 or higher; and 12 of the 13 loci (92%) had a CR of 50 or higher. In terms of genetic distance, most of the loci appeared to be located near the ends of chromosomes: 77% of the loci in a distal region, which accounts for only 5% of the chromosomal length, if we could assume that the distribution of crossovers is random. A casual examination of the mouse and human genetic maps reveals no restricted distribution of allozyme genes (21, 22). Another possibility is that the allozyme loci are randomly distributed along chromosomes, but the crossover sites are not. The nonrandom distribution of crossover sites is supported by cytogenetic observations. Chiasmata indeed tend to form near the centromeres in eggs of *M. lateralis* and produce "V" shaped bivalents, evident in Figure 1 as well as in previously published photographs of meiotic chromosomes (11). Therefore, our interpretation of both the recombination and chiasma data is that crossover sites in female *M. lateralis* are preferentially distributed in a recombination hot-region proximal to the centromere. At the molecular level, recombination does not occur randomly at any place along the DNA, and recombination sites (often referred to as hotspots) are determined by particular sequences, such as the Chi (GCTGGTGG) in *E. coli*, (AGGC)n in mouse, and the minisatellite sequences in mammals (23–25). However, the recombination hotspots identified so far account for only a small percentage of the total recombination events. The recombination hot-regions, as suggested by this study, could be chromosomal regions where the recombination hotspots are densely populated, or activated. It is not known whether the recombination hot-regions are related to heterochromatic regions.

Normally the nonrandom distribution of crossovers (or chiasmata) occurs where there are two or more chiasmata per chromosome, so that the position of the first chiasma affects the position of another one (chiasma interference). This study indicates that, even when there is only one chiasma per chromosome, this chiasma is still not randomly distributed along chromosomes. Most models for mechanisms of chiasma determination are based on chiasma interaction, including the most prevailing one—Fox's sequential model (12, 13). According to the sequential model, chiasma formation is sequential from telomere to centromere, and when there is only one chiasma, it is usually located near the telomere. In *M. lateralis*, the single chiasma is near the centromere.

The finding of only one crossover per chromosome with restricted distribution may explain the high levels of heterozygosity in *M. lateralis* and other marine bivalves (compared with most other animals), and the unique phenomenon of heterozygote deficiency (26, 27). In chromosomal regions having few crossovers, linkage disequilibrium would interfere with selection, resulting in the coexistence of numerous alleles at a locus. For example, if a deleterious allele at one locus is linked to a favorable allele at another, and the linkage is strong due to a lack of recombination, the deleterious allele could not be easily eliminated by selection, leading to high levels of heterozygosity and possibly heavy genetic loads. Further, genes within the linkage group could become coadapted

as a block, the survival of which would no longer be determined by the fitness of individual alleles, but would be dependent on encountering a similar, complementary, or coevolved block. If two blocks differed too much, they might produce heterozygotes which would be less fit than homozygotes. The coadapted complex may provide one explanation for heterozygote deficiency, but many other hypotheses have previously been advanced (27, 28, 29).

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